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Frequency of the K303R Mutation Stratified by Nodal Status

	<u>WT ER</u>	<u>MUT ER</u>
Axillary N-	16	10 (38%)
Axillary N+	4	23 (85%)
	—	—

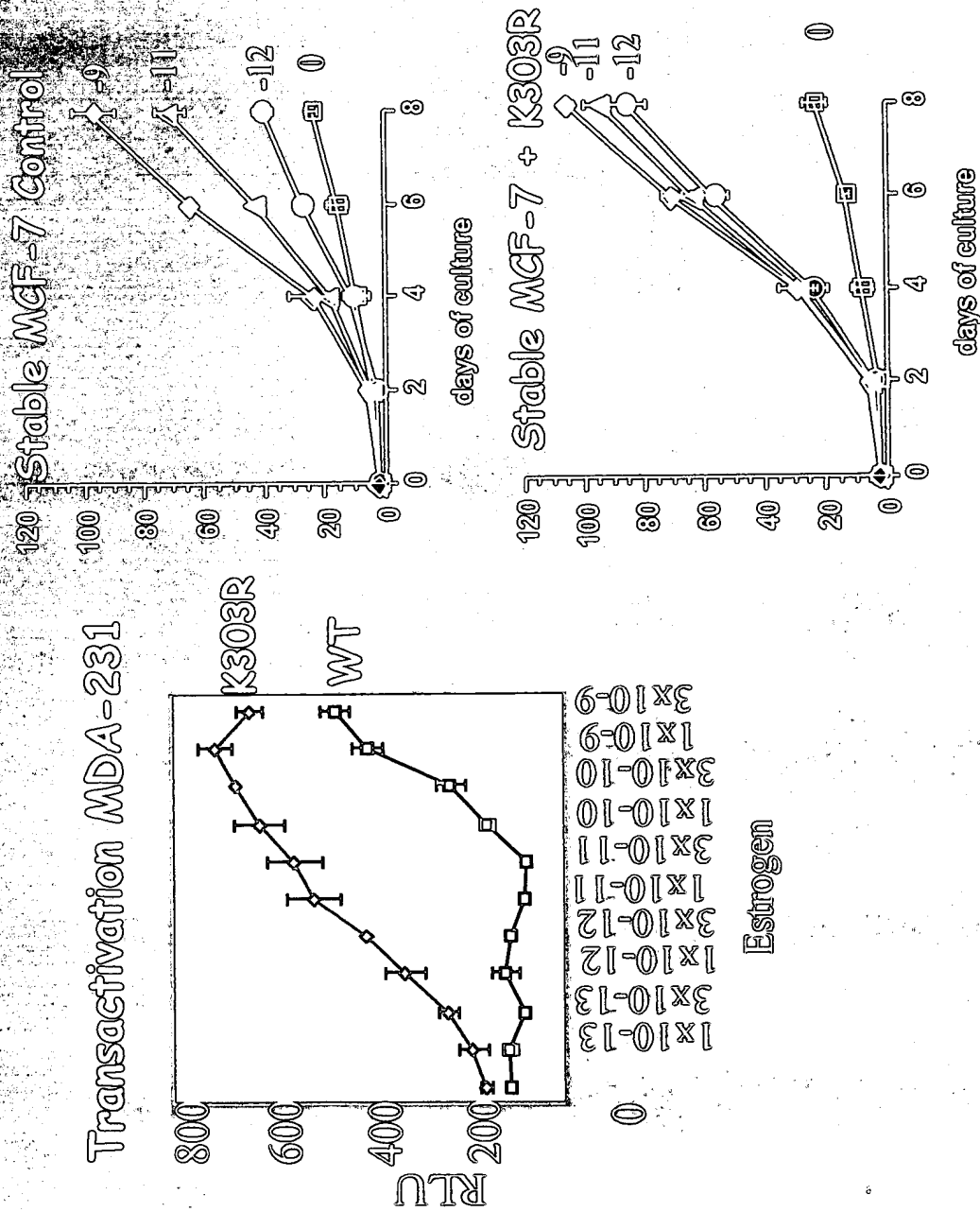
p=0.00062, Fisher's Exact Test

The Mutation is Associated with
Advanced Breast Cancer

Hypothesis:

Proliferative Advantage is due to Hypersensitivity to Estrogen

Growth Assay



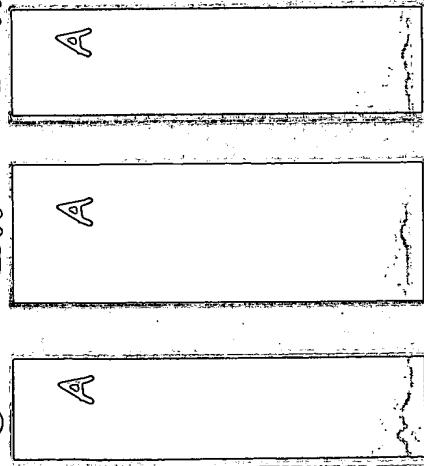
The K303R ERα Mutation is Frequently Present in Postmenopausal, ER-positive Patients

Neo-adjuvant study of 30 primary invasive breast cancers from UK^{***}:

Node-negative
Postmenopausal
Size > 2 cm
ERα-positive

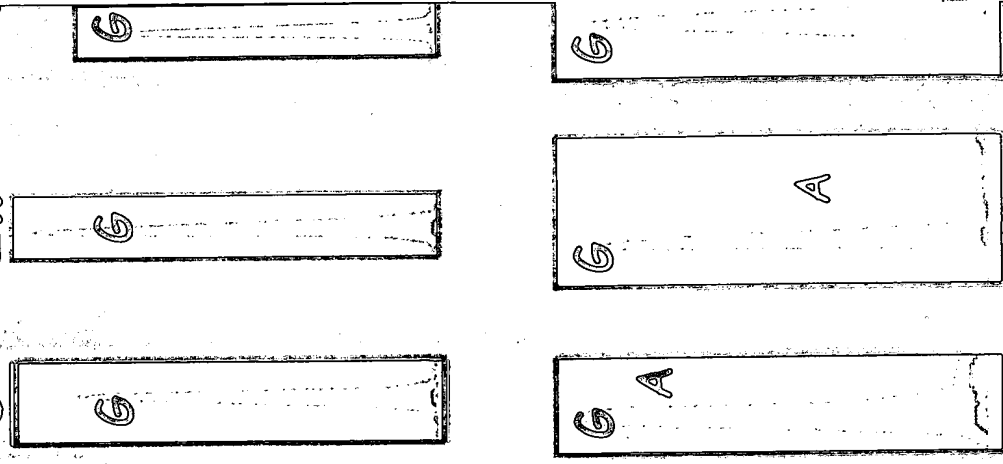
10% WT

0 → 2w → 12w



90% Mut

0 → 2w → 12w

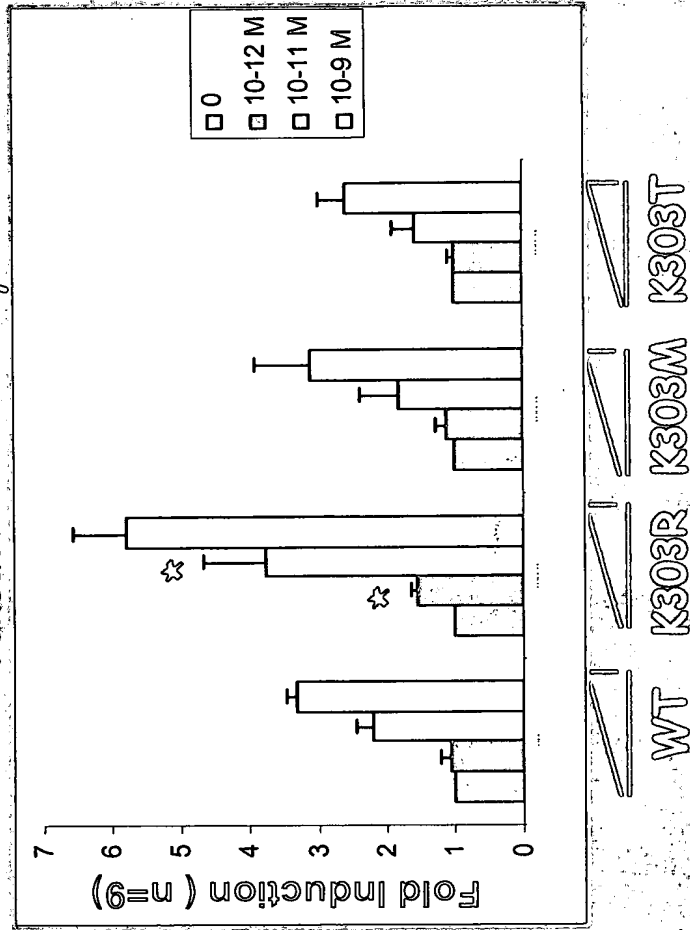


*** M. Dowsett

Why Find Only A to G Mutation in Tumors?

A to G = K303R
 A to T = K303M
 A to C = K303T

Transactivation Assay



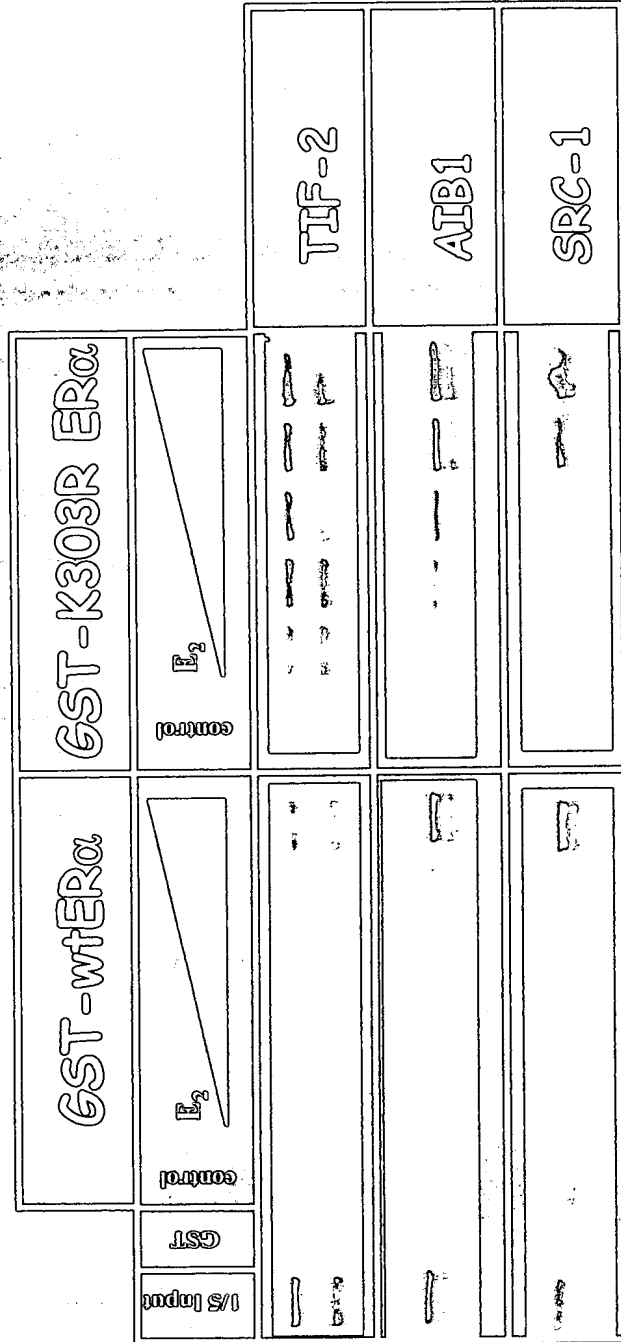
E₂

ERα

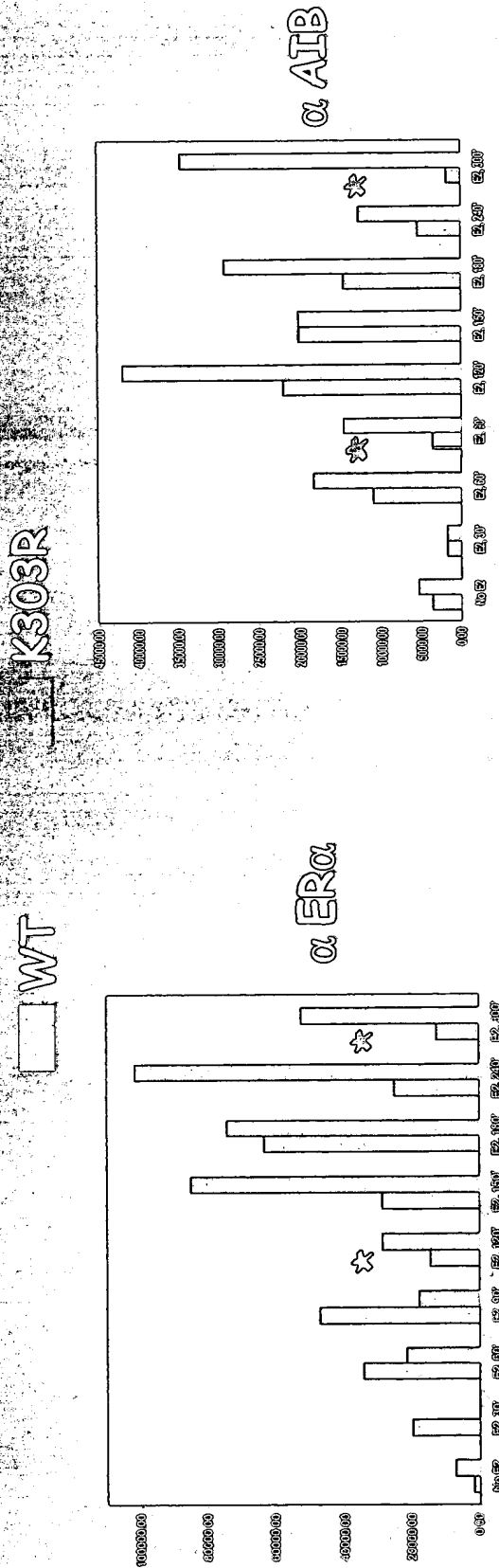
Only K303R is Hypersensitive

The ER α Mutation Exhibits Altered Binding to TIF-2 and AIB1 ER Co-activators

GST Pull-Down Assay



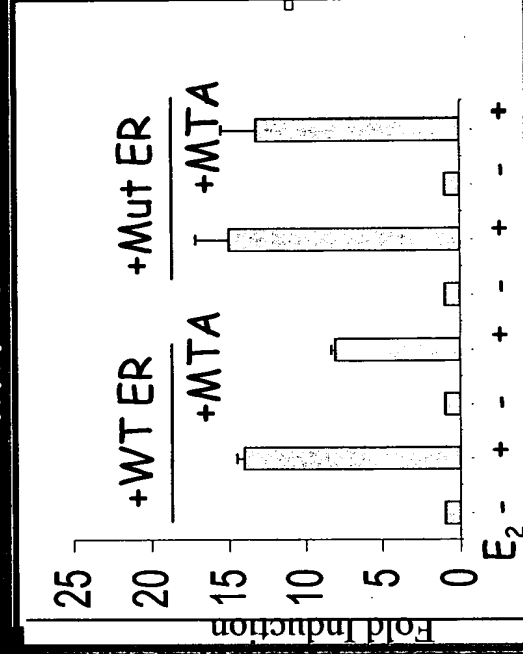
Altered Dynamics of K303R ER α on the pS2 Promoter



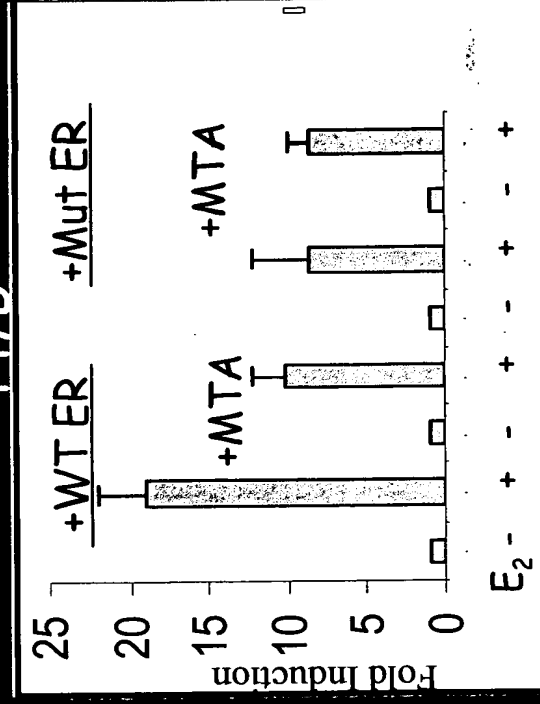
The K303R ER α Mutant is Resistant to MTA2 Co-Repressor Inhibition Of ER α Activity

Transactivation Assays

MCF-7



T47D

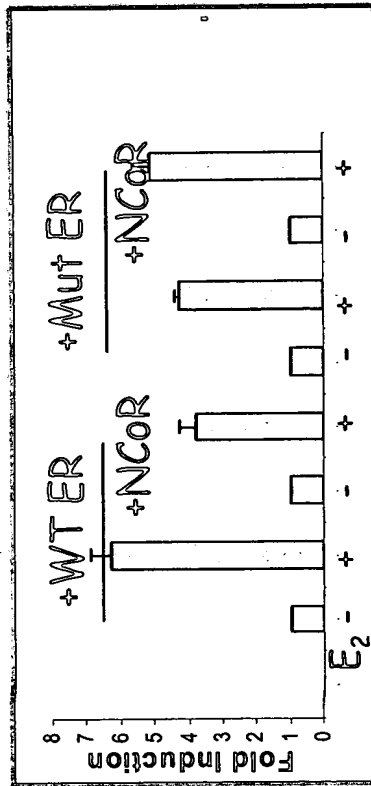


e K303R ER Mutant is Resistant to NCoR and BRCA1 Co-Repressor Inhibition of ERα Activity

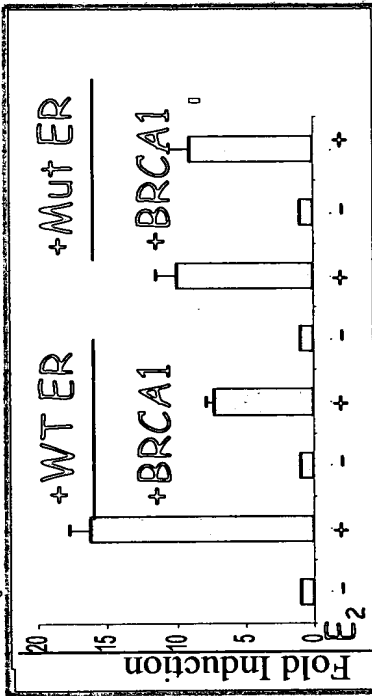
HeLa

Transactivation Assays

T47D



+NCoR



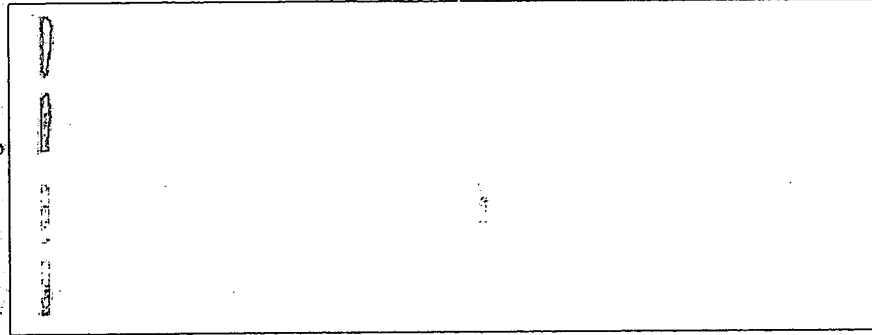
+BRCA1

The K303 ER α Site is Acetylated by p300

In Vitro Acetylation Assay

p300

autoacetylation



3, GATA-1 motif

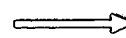
R X K K

Acetylated by HATs
(p300 & P/CAF)

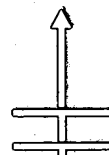
WT ER motif

R S K K

Acetylated by p300,
not P/CAF



Mutant 303 R



Acetylation by p300
is blocked

Wang et al., JBC. 2001

re Receptor Acetylation and Phosphorylation Coupled Events?

Acetylation

PO₄ ———

p53 or H3

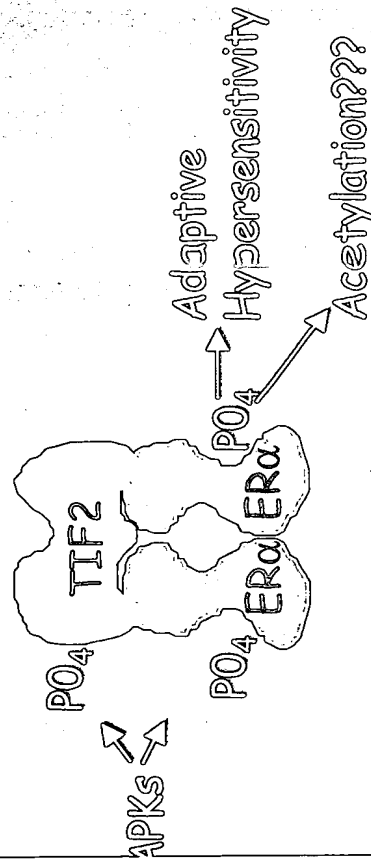
Total *In Vitro* Kinase Assay:
GST-ERs Hinge #253-310

+

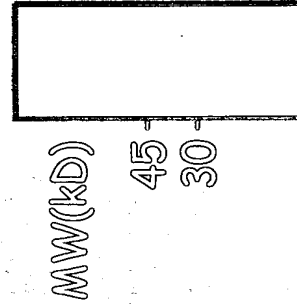
MCF-7 Lysate

+

γP32



WT
K303R

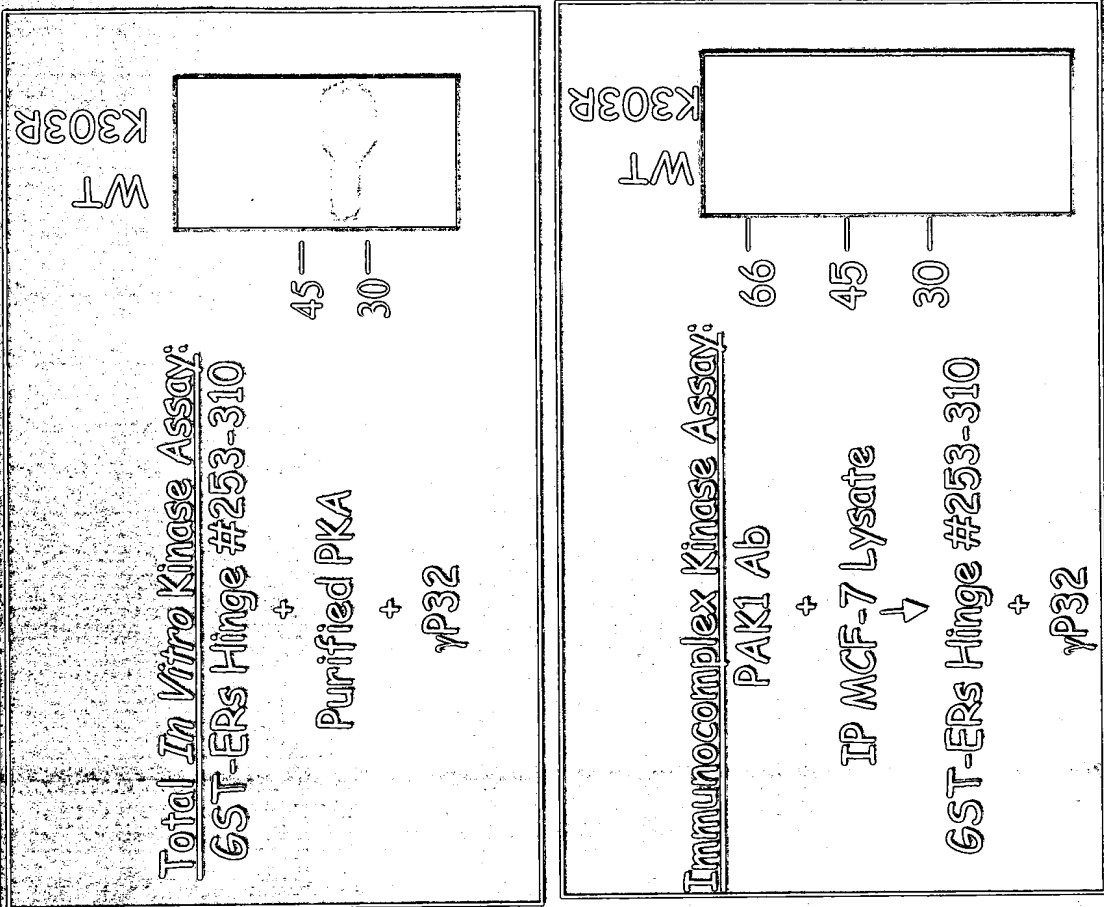


K303R ERα Mutation Generates an Efficient PKA & PAK1 Phosphorylation Site

Kinase	Substrate Specificity		
PKA	K/RXS (100%)	K/KXS (1%)	
PAK1	RXS (100%)	KXS (3%)	

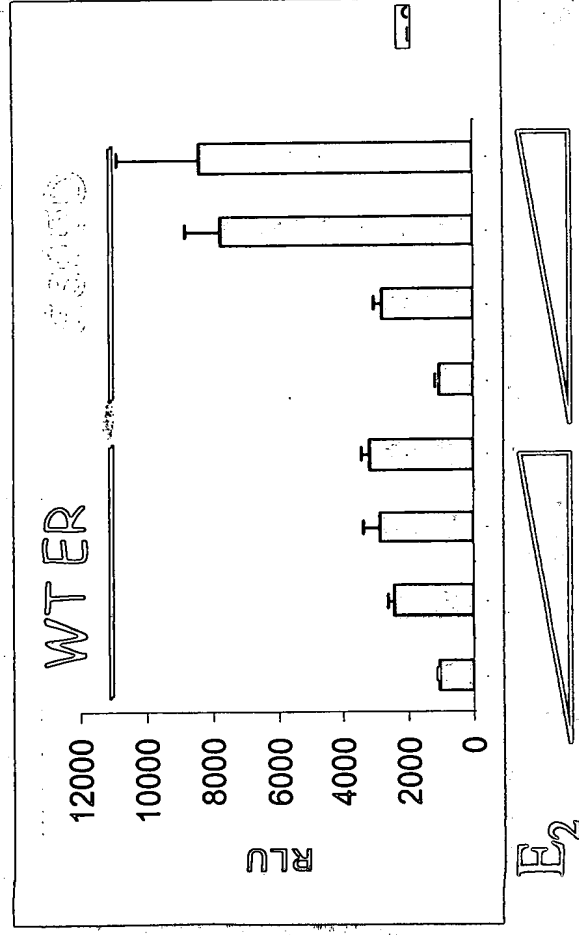
Tuazon, et al. Biochem 1997

WT ER:	KKNS ₃₀₅
K303R:	KRNS ₃₀₅



ERα S305 Phosphorylation Reduces Receptor Acetylation

Transactivation Assay



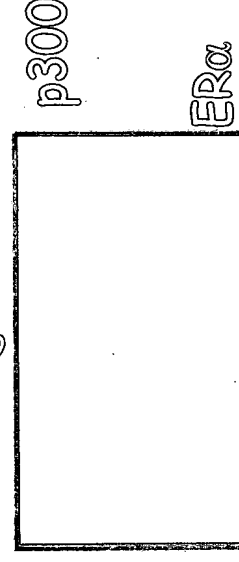
Mutant mimicking phosphorylation at S305 increases estrogen sensitivity

Acetylation Assay: GST-ERs (Hinge #253-310)



Acetylate with p300

GST-p300
GST-K303R
GST-WT
GST-K303R/S305A
GST-S305A
GST-K303R/S305D
GST-S305D



* Acetylation

Coomassie
blue stain

Other examples where phosphorylation blocks p300 acetylation:
Positive co-activator PC4, Forkhead TF FOXO 3a

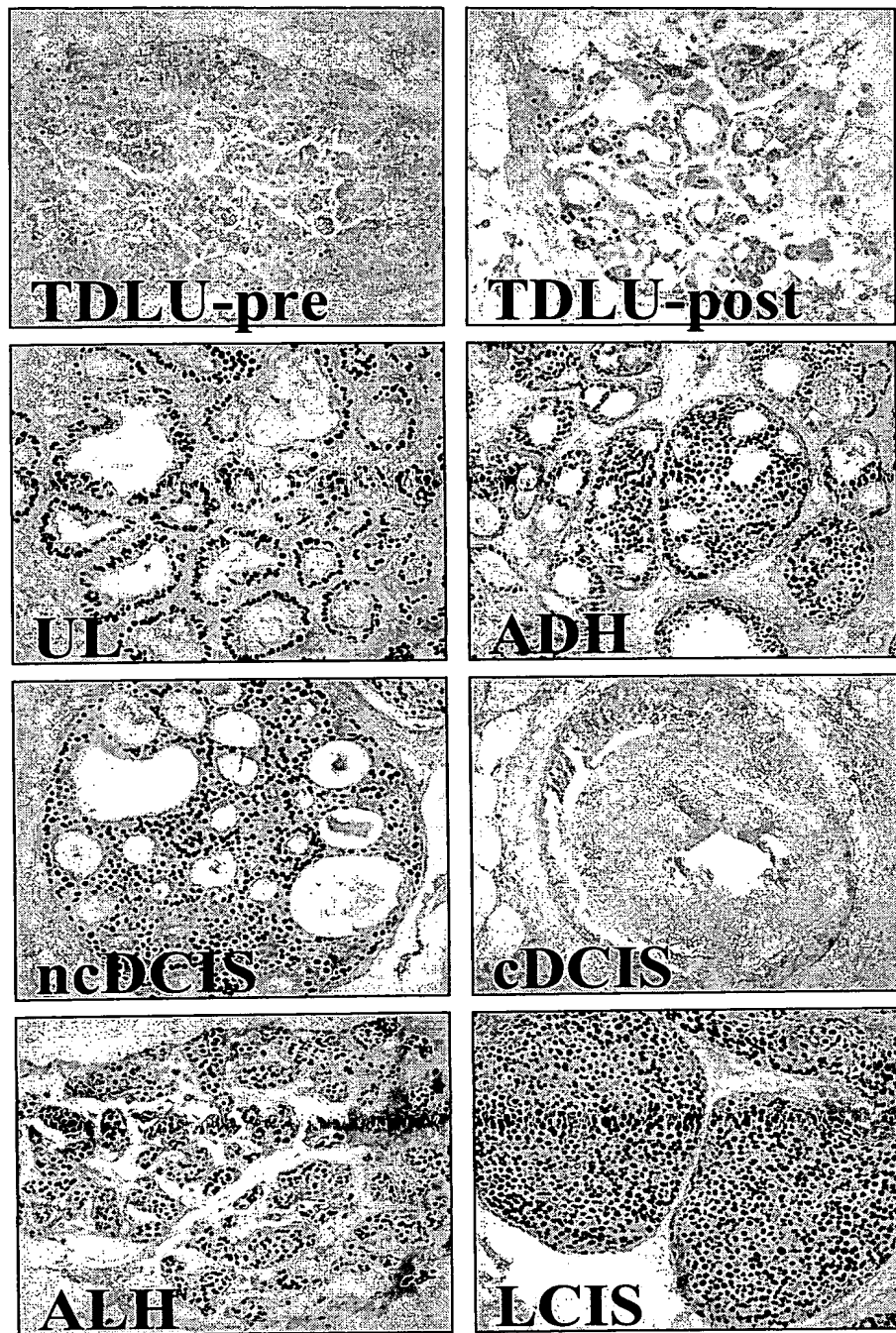


FIG. 1



REPLACEMENT DRAWING

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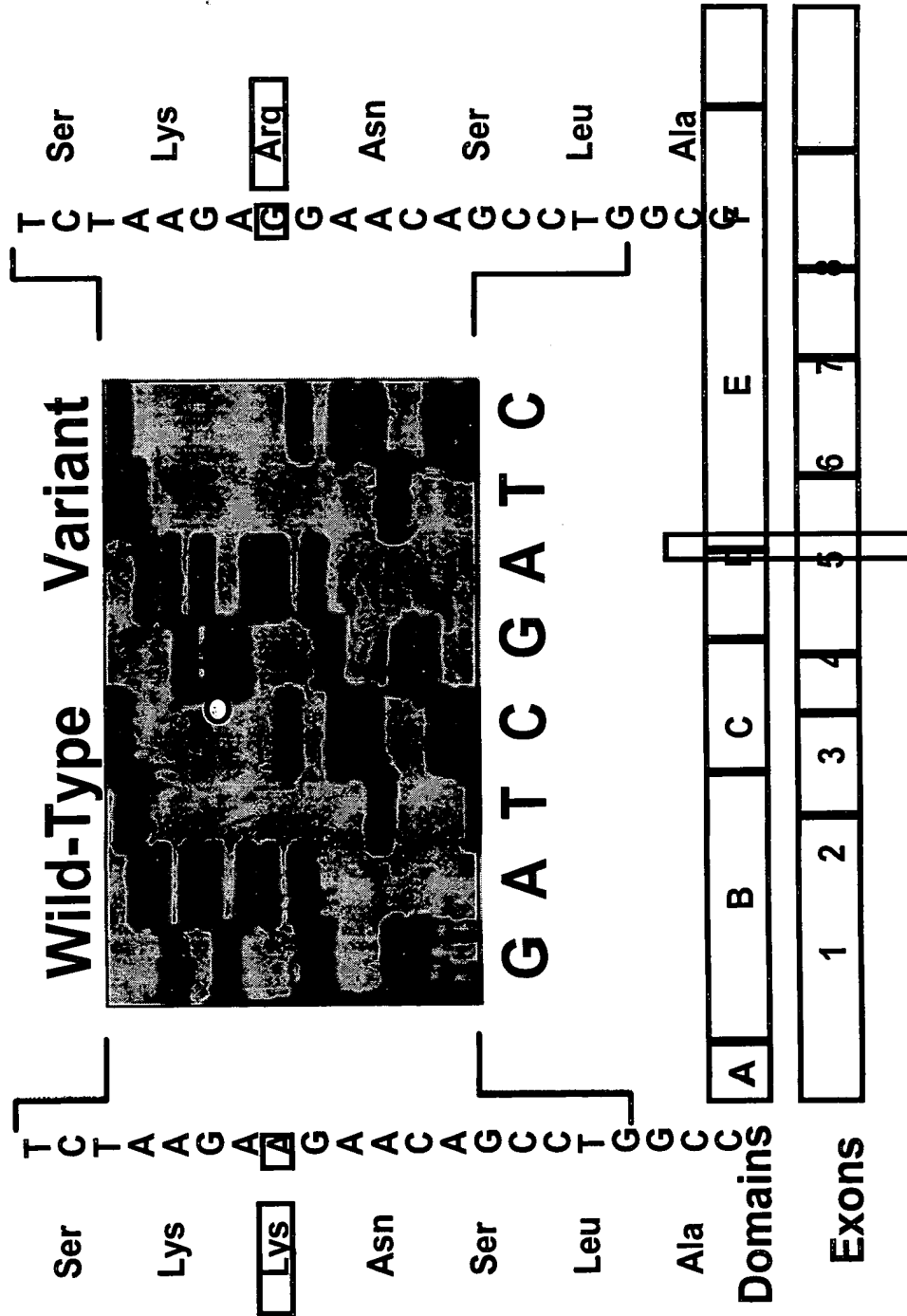


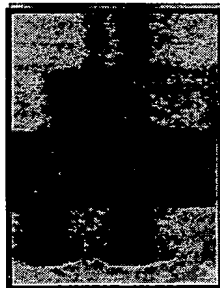
FIG. 2



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N Dis.

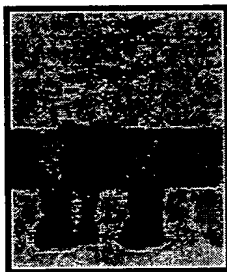
G A T C



WT

TH

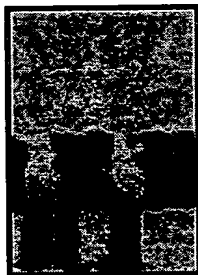
G A T C



VAR

N Adj.

G A T C



VAR

FIG. 3

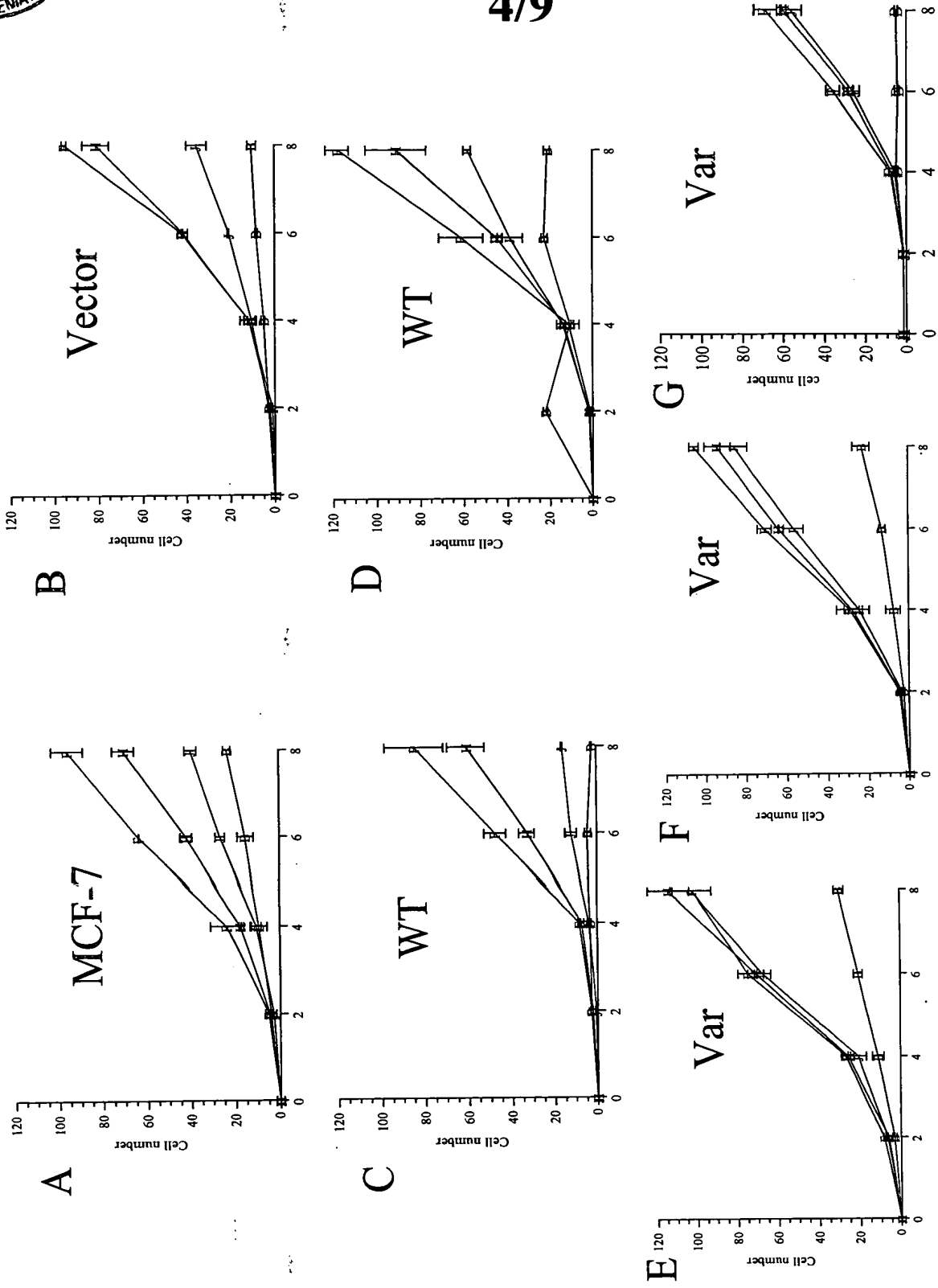


FIG. 4



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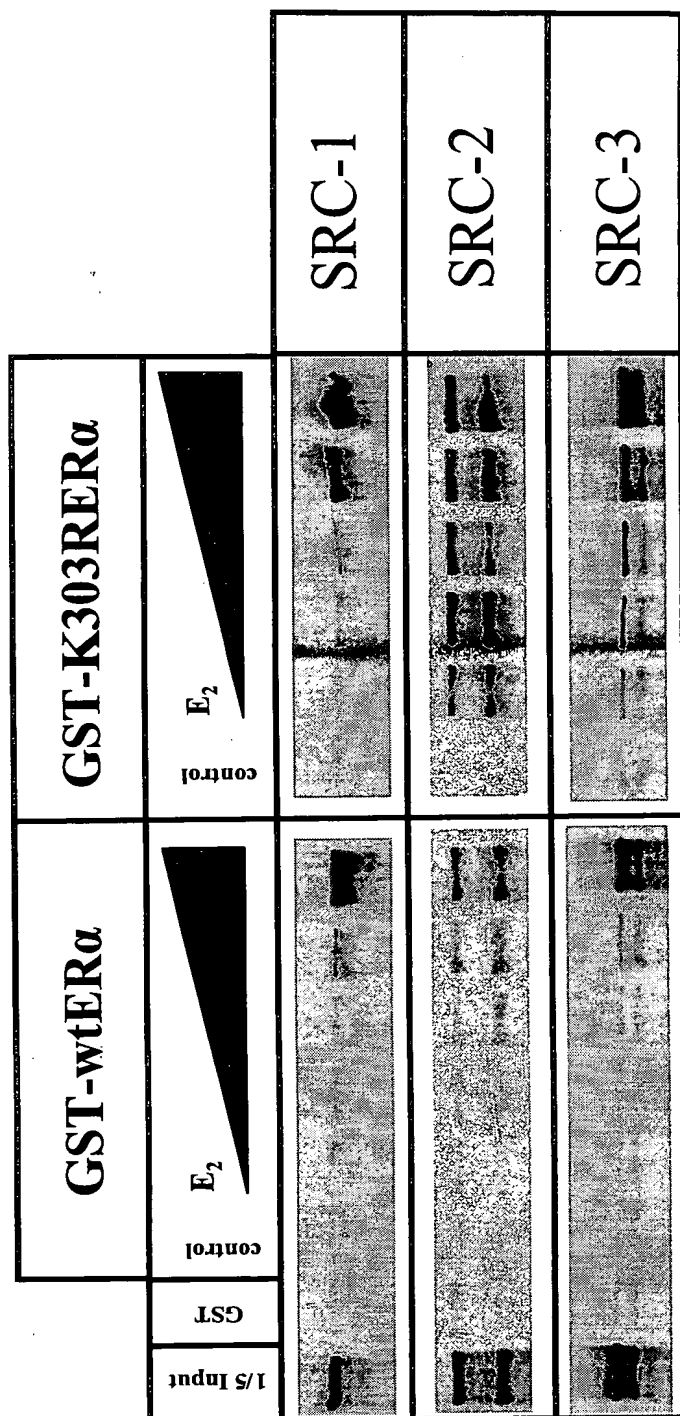


FIG. 5



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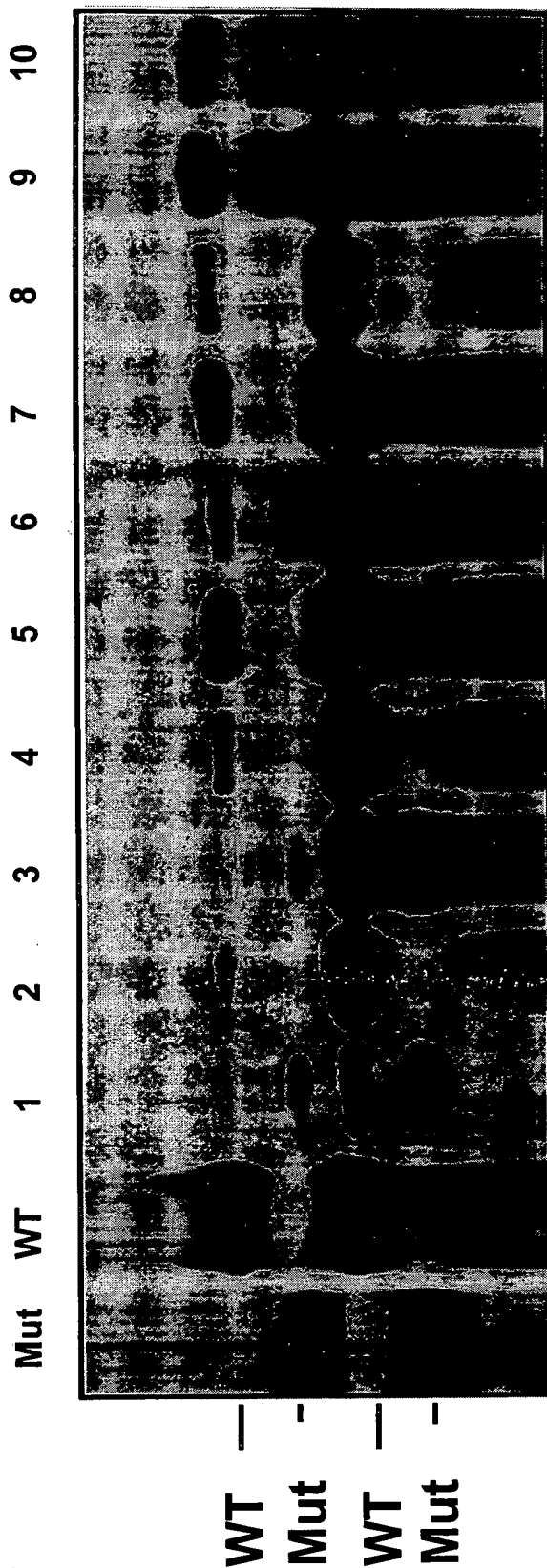


FIG. 6



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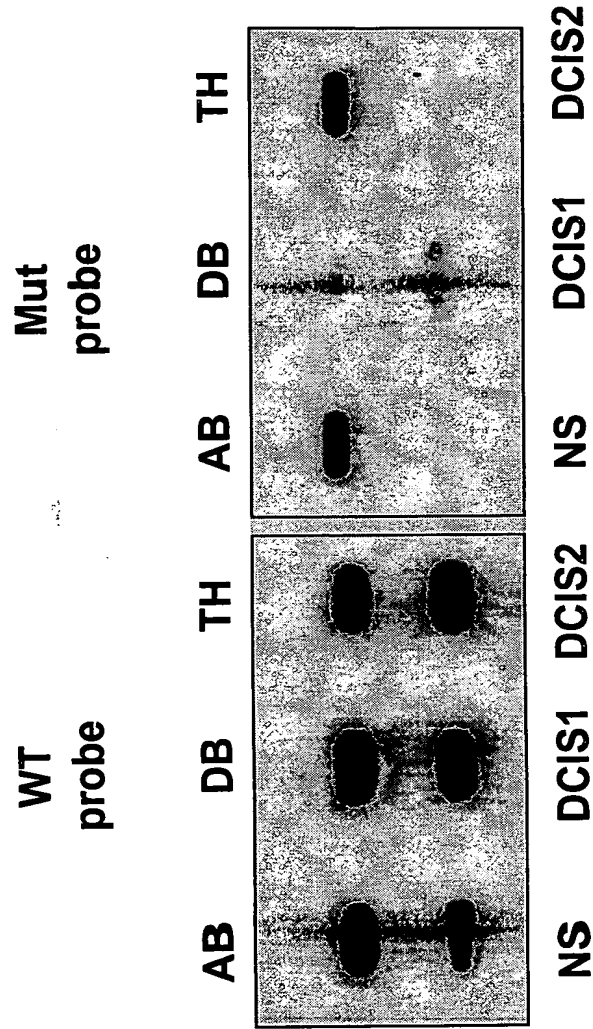
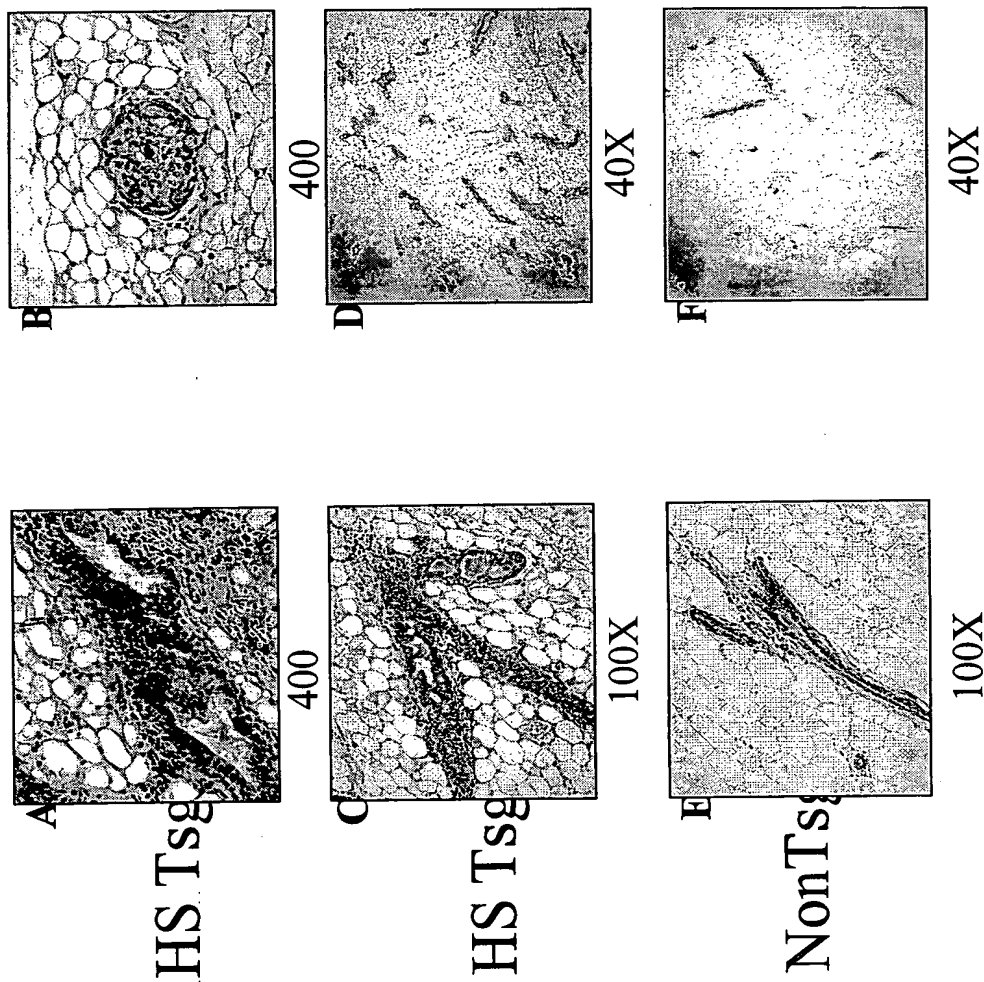


FIG. 7



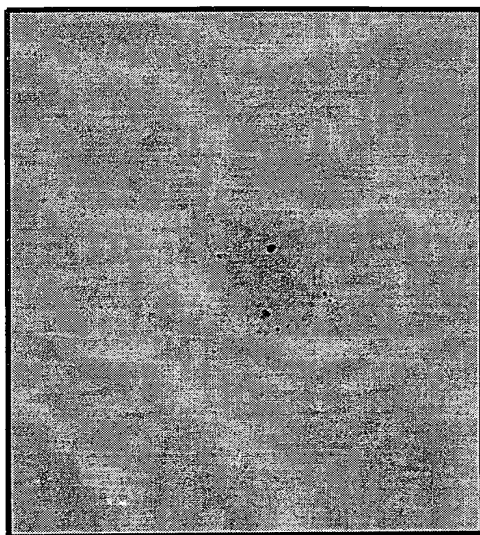
18 month old virgin mice-H&E

FIG. 8

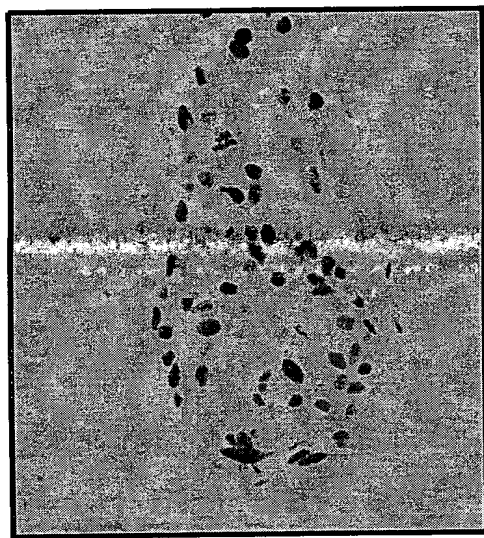


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NonTsg



K303R Tg



pH1b

18 month old virgin mice – pH1b IHC

FIG. 9